The function of a haptoglobin-haemoglobin receptor and the uses thereof

The present invention relates to haptoglobin-haemoglobin (Hp-Hb) complex or a part thereof or a mimic thereof being operably linked to a substance and capable of binding a CD163 receptor. Furthermore, the invention relates to a CD163 variant, membrane bound or soluble, capable of binding at least one haptoglobin-haemoglobin (Hp-Hb) complex, and the use of the Hp-Hb complex and the CD163 receptor for therapy.

Background of the invention

Normal adult haemoglobin consists of a tetramer of four haemoglobin chains, two α-chains and two β-chains. O_2 binds to the tetrameric form of haemoglobin and is transported in the blood. Fetal blood comprises fetal haemoglobin, a tetramer consisting of two α-chains and two γ-chains. Further haemoglobin chains have been identified, such as δ-chains, ε-chains, zeta-chains, τ-chains or the S form known to be the mutation seen in haemoglobin of individuals suffering from sickle cell disease.

Intravascular lysis of red blood cells (haemolysis) leads to the release of haemoglobin into plasma. This phenomenon occurs during physiological as well as pathological conditions. Pathological complications are severe when accelerated in infectious e.g. malaria), inherited (e.g. sicle cell anemia), or autoimmune diseases. The haemoglobin tetramers are converted to haemoglobin dimers capable of binding haptoglobin. In the plasma haemoglobin is captured by the acute phase protein haptoglobin. Haptoglobin is a blood plasma protein having a molecular weight of approximately 86.000 to 400.000 and plays an important role in the metabolism of haemoglobin liberated into the blood stream. When liberated excessively in the blood the haemoglobin is excreted into the urine through the renal tubules, resulting in not only an iron loss but also disorders of the renal tubules. Because haptoglobin binds selectively and firmly to haemoglobin in vivo and thereby forms a haemoglobin-haptoglobin complex, it has important functions in the recovery of iron and in the prevention of renal disorders.

Hp is synthesised as a single chain, which is post-translationally cleaved into an amino-terminal α chain and a carboxy-terminal β chain. The basic structure of Hp, as found in most mammals, is a homodimer (Fig. 2a), in which the two Hp molecules are linked by a single disulfide bond via their respective ~9 kDa α chains. In man, a variant with a long α chain is also present in all populations. This variant arose apparently by an early intragenic duplication, presumably originating from an unequal crossover of two basic alleles, resulting in an Hp with an α chain of ~14 kDa. The short and long α chains are designated as α^1 and

α^2 respectively. Since the cysteine forming the intermolecular disulfide bond between the α chains is also duplicated, humans carrying the long variant allele exhibit a multimeric Hp phenotype (Fig. 2a).

Conventional human haptoglobins have been well studied; they were discovered over 40 years ago and their role is thought to be in the plasma transport of free haemoglobin. Additionally, haptoglobin is believed to have anti-inflammatory activities, such as its decreasing effect on neutrophil metabolism, and an effect on the immune system by possibly modulating B cell proliferation and decrease antibody production. The mechanisms of the influence of haptoglobin on immune function is unknown. The potential signalling pathways by which haptoglobin is mediating its effects, and the existence of a haptoglobin receptor have not been disclosed in the prior art.

However, Ghmati et al., 1996 describe a study in which haptoglobin is an alternative low-affinity ligand for CD11b/CD18 on monocyte cell lines. CD11b/CD18 is part of the integrin family and is involved in inflammatory and immunological functions.

Yet another receptor molecule present on monocytes is CD163. It is identified as a member of the scavenger receptor cystein-rich superfamily (SRCR) present on cells of the monocytic family, such as most macrophages. Ritter et al., 1999 discuss the regulation, promoter structure and genomic organisation of the CD163 receptor. The precise function of CD163 is not disclosed. Furthermore, previous work on the biological function of CD163 is limited to a study on the effect of antibody-mediated crosslinking of CD163 on cultured monocytes(Van den Heuvel, M.M. et al. Regulation of CD163 on human macrophages: cross-linking of CD163 induces signalling and activation. J. Leukoc.Bil. 66, 858-866 (1999). The CD163 surface ligation induces a tyrosine kinase dependent signal resulting in intracellular calcium mobilisation, inositol triphosphate production, and increased secretion of anti-inflammatory cytokines.

Summary

The present inventors have identified CD163 as the high-affinity macrophage receptor for haptoglobin-haemoglobin complexes. They also have identified a soluble form of CD163 in plasma of normal human subjects and found a correlation between membrane bound and soluble receptor. Under normal conditions approx. 100-500 µg/l soluble CD163 is present in plasma. The present invention relates to the use of the CD163 receptor, membrane bound or soluble and/or a CD163 variant, and/or the use of haptoglobin-haemoglobin complexes in the diagnosis, prevention and/or treatment of various diseases and disorders.

Accordingly, the invention describes a Hp-Hb complex, or a part thereof or a mimic thereof being operably linked to a substance, wherein the Hp-Hb complex is capable of binding CD163 and/or a CD163 variant. In the present context the term Hp-Hb complex includes a functional equivalent thereof unless expressively otherwise stated.

In the present context the term "substance" means a component heterologous to the Hp-Hb complex, such as a drug, a gene, a vesicle, a vector, or the like.

Further, the invention concerns the use of at least one Hp-Hb complex for the delivery of at least one drug, or at least one gene to a cell expressing a CD163 receptor and/or a CD163 receptor variant. The invention also relates to the use of at least one Hp-Hb complex, further comprising a CD163 receptor variant for the identification of at least one Hp-Hb complex in serum and/or plasma of an individual.

In the present context the term CD163 receptor covers both the conventional scavenger receptor CD163 of monocytes and most tissue macrophages as well as the soluble form of CD163, sHbSR unless otherwise specified. The term CD163 is used synonymously with the term CD163 receptor. The term sHbSR is used interchangeably with soluble CD163 receptor.

The term a CD163 receptor variant is used synonymously with the term CD163 variant.

In another aspect, the present invention relates to a CD163 variant capable of binding at least one haptoglobin-haemoglobin (Hp-Hb) complex.

In a further aspect of the invention the use of at least one CD163 variant in the manufacture of a medicament for treatment of disorders/complications related to haemolysis in an individual in need of such treatment is disclosed.

Also, the invention describes the use of at least one CD163 variant for the removal of at least one Hp-Hb complex in serum and/or plasma of an individual, and the use for the determination of the haemolysis rate of an individual. Further, the use of at least one complex comprising haemoglobin and haptoglobin as a marker for a cell expressing a CD163 variant, wherein at least one of the haemoglobin or haptoglobin molecules are labelled is also described in the present invention.

An object of the invention is to provide a CD163 molecule for the use as a medicament. The areas of use of a CD163 molecule according to the invention are identical to the areas of use described above for the CD163 variant.